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THE CORRELATION OF CERTAIN REACTIONS OF COLON BACILLI AND LIKE ORGANISMS WITH SOURCE *

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The colon-aerogenes group of bacteria has claimed the interest of bacteriologists from the discovery of the first member of the group until the present time, altho opinion as to the exact meaning of their presence in water and milk has changed somewhat. Since these organisms are known to occur in the intestines of all animals, their presence in either milk or water was at first regarded as an evidence of fecal contamination. Later Prescott and Winslow[1] regarded the number of the organisms present as more significant than their mere presence. Be this as it may, since there is always a possibility that they may indicate pollution, it is of advantage to have quick methods of determining whether or not organisms present in milk or water belong to the colon group, and if possible their source.

Much work has been done on the classification of the group in England, Germany, and America altho most of this work has been along the line of differentiating different members of the group by their powers of fermentation in carbohydrate media. MacConkey[2] finally narrowed his classification down to 4 groups, separated from each other on the basis of their power to ferment saccharose and dulcite. In connection with their fermenting powers he considered other characteristics, such as gelatin-liquefaction, indol-production, motility, and the Voges-Proskauer[3] reaction. Twort,[4] Vorloud,[5] and Winslow and Walker[6] also differentiated this group on the basis of their fermenting powers in carbohydrate media.

Bergey and Deehan[7] isolated a large number of organisms from milk, kefir, and sewage, and studied them with a view to classifying them according to the scheme of MacConkey. They divided the whole group into 16 subgroups according to ability to ferment saccharose, dulcite, adonite, and inulin, and each of these again into 16 smaller groups according to motility, gelatin-liquefaction, indol-production, and the Voges-Proskauer reaction. It

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[1] Elements of Water Bacteriology, 1915, p. 149.

[2] Jour. Hyg., 1905, 5, p. 333.

[3] Ztschr. f. Hyg. u. Infektionskrankh., 1898, 28, p. 20.

[4] Centralbl. f. Bacteriol., R., 1907, 40, p. 508.

[5] Ibid., I, O., 1908, 45, p. 97.

[6] Science, 1907, 26, p. 797.

[7] Jour. Med. Research, 1908, 14, p. 175.

remained for Rogers, Clark, and Davis[8] to utilize a method first used by Keyes[9] to determine accurately the amount of gas evolved by different strains of colon bacilli and to arrange them into classes according to this.

Keyes found that colon bacilli grown on synthetic media and in vacuo produce a definite volume of gas in a given time and that the ratio of $CO_2:H_2$ is constant. Rogers, Clark, and Davis repeated this work of Keyes and with slight modifications divided the colon bacilli into classes according to their $CO_2:H_2$ ratios. They found that all the organisms fell into 3 groups: a high-ratio group, in which the $CO_2:H_2$ ratio is 1.90 or above this; a low-ratio group, in which the ratio is about 1.06; and an ∞ ratio group, in which only CO_2 is evolved. Their next step was to make a series of investigations of like organisms from different known sources to see if those obtained from any one source could be definitely related to any one of these gas ratios. In their system of classification, the gas ratio formed the only basis of differentiation; members classed within a single group display marked differences in powers of liquefaction, indol-production, and motility, but all agree in gas ratios.

Rogers, Clark, and Evans[10] made a study of 150 strains from bovine feces and found that 149 of them showed a low gas ratio. Rogers studied systematically organisms like the colon bacilli, from surface water, and found that one-third of these organisms corresponded to the type found in human and bovine feces. Rogers, Clark, and Evans[11] in an extensive study of the colon group occurring on grains found that the majority of these organisms belonged to the high-ratio group and that none of those found on grain corresponds to those found in feces.

Routine gas analysis in vacuo, however, is too complicated and requires too much apparatus, presenting technical difficulties not easily overcome in the average bacteriologic laboratory, and so any detectable characteristic which could be found invariably accompanying gas-production would prove a boon. This was found by Clark and Lubs[12] in the use of two easily accessible indicators. They found that when the organisms, in producing gas, had reached the maximal amount, the medium had a certain H^+ ion concentration and that this could be measured by the use of either methyl red or paranitrophenol (preferably methyl red), as this H^+ ion concentration happens to be within the limits of their color changes. Paranitrophenol is colorless in a H^+ ion concentration of about 1×10^{-5}; in a concentration below this it gradually becomes colored till at a concentration of $H^+1 \times 10^{-6}$ it is yellow-green. Methyl red is even more brilliant as an indicator, being bright red at a H^+ ion concentration of 1×10^{-5} and changing to clear yellow at a H^+ ion concentration of $H^+1 \times 10^{-6}$. The high-ratio cultures give a green color with paranitrophenol and a yellow color with methyl red, indicating that they are alkaline, while the low-ratio cultures give no color with paranitrophenol and a red color with methyl red, indicating that they are acid.

It was found by these investigators that only those organisms isolated from feces produce this low ratio, so that the sanitary significance is easily seen, it being only necessary to plant the organisms in the peptone dextrose dihydrogen-phosphate medium, incubate for 5 days at 30 C., and then determine the

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[8] Jour. Infect. Dis., 1914, 14, p. 411.

[9] Jour. Med. Research, 1909, 21, p. 69.

[10] Jour. Infect. Dis., 1914, 15, p. 99.

[11] Ibid., 1915, 17, p. 137.

[12] Ibid., p. 160.

H^+ ion concentration. Levine[13] in a recent paper correlated the Voges-Proskauer reaction with the H^+ ion concentration, having found that only those organisms that show a high gas ratio give the Voges-Proskauer reaction, and that these are rarely found in feces.

It might be interesting to note in this connection that at the meeting of the Society of American Bacteriologists in Urbana, Ill., in 1915, Winslow and Kligler[14] reported on the standard tests for colon bacilli, the committee, previously appointed, having decided on (1) titratable acidity, (2) H^+ ion concentration, (3) milk reactions, (4) indol-production, (5) gelatin liquefaction, (6) chromogenesis. This gives 3 large groups; (1) B. coli—coagulating milk, high H^+ ion concentration, indol-positive; (2) B. aerogenes—coagulating milk, low H^+ ion concentration, indol-negative; and (3) the B. typhi group—alkaline reaction, high H^+ ion concentration. There are 3 subgroups to Group 1 according to indol-production and sucrose-fermentation and 3 subgroups to Group 3.

A number of organisms which had been isolated and previously studied in this laboratory were selected for reinvestigation and these were studied according to the different methods.

TECHNIC

The fermentative powers were tried with lactose, sorbite, raffinose, dextrin, dextrose, saccharose, dulcite, mannite, maltose, adonite, and inulin, a Hiss serum-water medium containing 1% of the various carbohydrates being used.

The Voges-Proskauer reaction was studied according to Levine's method, the Clark and Lubs medium* for determining H^+ ion concentration being used, with an incubation of 48 hours, after which 5 c.c. of a 10% KOH solution were added. All cultures were kept 1 week as some of them became positive in that time.

For liquefaction of gelatin the gelatin was inoculated with a few drops of a broth culture and the level of the surface of the gelatin marked by a narrow strip of paper. The tube was allowed to stand 30 days. The liquefaction was measured in millimeters.

Indol-production tests were made by inoculating peptone and incubating for 7 days, then adding H_2SO_4 drop by drop, and finally 1 c.c. of 0.01% $NaNO_2$ solution.

The indicator tests were made as described by Clark and Lubs.

The fermentative powers of colon bacilli from milk, which had been tried previously by Dr. Bergey, in some cases seemed to have been lost as regards certain carbohydrates, as I could not obtain fermentation with numerous tests and on different lots of the same kinds of media. No. 2, which was found by Dr. Bergey to belong to the group fermenting saccharose, dulcite, adonite, and inulin, seemed to have lost all power by the time my work was undertaken. No. 3 had lost the power to ferment lactose and sorbite; No. 8, the power to ferment

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[13] Jour. Infect. Dis., 1916, 18, p. 358.

[14] Jour. Bacteriol., 1916, 1, p. 81.

* Witte's peptone 0.5%, dextrose 0.5%, K_2HPO_4 0.5% are heated in 800 c.c. of water and stirred for 20 minutes; the whole is then filtered, cooled to 20 C., diluted to 1,000 c.c., and tubed. The medium is sterilized by the intermittent method.

TABLE 1

Fermentative Powers of Colon Organisms from Various Sources

Number	Lactose	Sorbite	Dextrin	Dextrose	Saccharose	Dulcite	Mannite	Maltose	Adonite	Inulin	Raffinose
From Milk											
1	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	+	−	−	+	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−
5	+	+	−	+	−	−	+	+	−	−	−
6	−	−	+	+	+	−	−	+	−	−	−
7	+	+	−	−	+	+	+	+	−	−	+
8	+	−	−	−	−	−	−	−	−	−	−
9	+	+	+	−	−	−	+	−	−	−	+
10	+	−	+	+	+	−	+	+	−	−	+
11	+	+	+	+	+	−	+	+	−	−	+
12	+	+	−	+	−	−	+	+	−	−	−
13	+	+	−	+	+	+	+	+	−	−	+
14	+	+	+	+	+	+	+	+	−	−	+
15	−	−	−	−	−	−	−	−	−	−	−
16	+	−	−	+	+	−	+	−	−	−	−
17	+	+	−	+	−	−	+	+	+	−	−
18	+	+	−	+	+	+	+	+	−	−	+
From Human Feces											
1	+	+	+	+	+	+	+	+	−	−	+
2	+	+	−	+	+	−	+	+	−	−	+
3	+	+	−	+	−	+	+	+	−	−	−
4	+	+	−	+	+	−	+	+	−	−	−
5	+	+	−	+	−	−	+	+	+	−	+
6	+	+	−	+	−	−	+	+	+	−	−
7	+	+	−	+	+	−	+	+	+	−	−
8	−	−	−	+	−	−	−	+	+	−	+
9	+	−	−	+	−	−	−	+	+	−	+
10	−	−	+	+	−	−	+	+	+	−	+
From Rabbit Feces											
1	+	+	−	+	+	−	+	+	−	−	+
2	+	+	+	+	+	+	+	+	−	−	+
From Water											
1	+	+	−	+	+	−	+	+	+	−	+
2	+	+	−	+	+	−	+	+	−	+	−
3	+	+	+	+	+	−	+	+	−	+	+
4	−	−	−	−	−	−	−	−	−	−	−
From Sewage											
1	+	+	+	+	+	−	+	+	−	−	+
2	−	−	−	−	−	−	−	−	−	−	−
3	+	+	−	+	+	−	−	+	−	+	−
4	−	+	−	+	+	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−	−
From Human Urine (Pyolitis)											
1	+	+	−	−	+	−	+	+	−	−	+
2	−	−	+	+	+	−	+	+	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−
From Egg Powder											
1	+	−	+	+	+	−	+	−	−	−	−
2	+	+	+	−	+	+	+	+	+	−	+
3	−	+	−	+	+	−	−	+	−	−	−

TABLE 2

OTHER PROPERTIES OF COLON ORGANISMS FROM VARIOUS SOURCES

Number	Voges-Proskauer Reaction	Liquefaction of Gelatin in Mm.	Indol-Production	P— Nitro-phenol	Methyl Red
FROM MILK					
1	+	3	—	Alkaline	Alkaline
2	+	0	—	Alkaline	Alkaline
3	+	40	+	Alkaline	Alkaline
4	+	5	—	Alkaline	Alkaline
5	—	5	—	Acid	Acid
6	+	40	+	Alkaline	Alkaline
7	+	0	+	Alkaline	Alkaline
8	+	0	+	Alkaline	Alkaline
9	+	0	+	Alkaline	Alkaline
10	+	0	+	Alkaline	Alkaline
11	+	0	+	Alkaline	Alkaline
12	—	0	+	Acid	Acid
13	+	0	—	Alkaline	Alkaline
14	+	15	+	Alkaline	Alkaline
15	+	3	+	Alkaline	Alkaline
16	—	10	—	Acid	Acid
17	—	0	—	Acid	Acid
18	—	0	+	Acid	Acid
FROM HUMAN FECES					
1	—	0	+	Acid	Acid
2	—	0	+	Acid	Acid
3	—	0	+	Acid	Acid
4	—	0	—	Acid	Acid
5	—	4	+	Acid	Acid
6	—	0	+	Acid	Acid
7	—	0	+	Acid	Acid
8	—	0	+	Acid	Acid
9	—	0	+	Acid	Acid
10	—	2	+	Acid	Acid
FROM RABBIT FECES					
1	—	0	+	Acid	Acid
2	—	0	+	Acid	Acid
FROM WATER					
1	—	10	+	Acid	Acid
2	—	20	—	Acid	Acid
3	+	8	—	Alkaline	Alkaline
4	—	4	+	Acid	Acid
FROM SEWAGE					
1	+	8	..	Alkaline	Alkaline
2	+	0	—	Alkaline	Alkaline
3	+	35	..	Alkaline	Alkaline
4	—	0	..	Acid	Acid
5	+	0	—	Alkaline	Alkaline
FROM HUMAN URINE					
1	—	0	+	Acid	Acid
2	—	13	—	Acid	Acid
3	+	0	—	Alkaline	Alkaline
FROM EGG POWDER					
1	+	0	+	Alkaline	Alkaline
2	+	0	+	Alkaline	Alkaline
3	+	0	—	Alkaline	Alkaline

TABLE 3

Classification of Colon Bacilli According to Bergey and Deehan

Source	S— D— A— I—	S+ D— A— I—	S— D+ A— I—	S— D— A+ I—	S— D— A— I+	S+ D+ A— I—	S+ D— A+ I—	S+ D— A— I+	S— D+ A+ I—	S— D+ A— I+	S— D— A+ I+	S+ D+ A+ I—	S+ D+ A— I+	S+ D— A+ I+	S— D+ A+ I+	S+ D+ A+ I+	Total
Milk	9	4	0	1	0	4	0	0	0	0	0	0	0	0	0	0	18
Human feces	0	2	1	5	0	1	1	0	0	0	0	0	0	0	0	0	10
Rabbit feces	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Water	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	4
Sewage	2	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	5
Urine	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Egg powder	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3
Total	13	13	1	6	0	6	2	3	0	0	0	0	1	0	0	0	45

S = Saccharose; D = Dulcite; A = Adonite; I = Inulin.

TABLE 4

Distribution of Colon Organisms Among MacConkey's Groups

Source	B. Acidi-Lactici Saccharose — Dulcite —	B. Coli-Communis Saccharose — Dulcite +	B. Coli-Communior Saccharose + Dulcite +	B. Lactis-Aerogenes Saccharose + Dulcite —	Total
Milk	10	0	4	4	18
Human feces	5	1	1	3	10
Rabbit feces	0	0	1	1	2
Water	1	0	0	3	4
Sewage	3	0	0	2	5
Urine	1	0	0	2	3
Egg powder	0	0	1	2	3
Total	20	1	7	17	45

TABLE 5

Correlation Between the Source of Colon Organisms and Saccharose-Fermentation

Source	Saccharose +		Saccharose —	
	Number of Organisms	Percentage	Number of Organisms	Percentage
Milk	8	44	10	56
Human feces	4	40	6	60
Rabbit feces	2	100	0	0
Water	3	75	1	25
Sewage	2	40	3	60
Urine	2	67	1	33
Egg powder	3	100	0	0

TABLE 6

Correlation of the Voges-Proskauer and Methyl-Red Reactions with Source

Source	Reaction to Methyl Red, 5 Days' Incubation		Voges-Proskauer Reaction	
	Acid or Neutral	Alkaline	Positive	Negative
Milk	5	13	13	5
Human feces	10	0	0	10
Rabbit feces	2	0	0	2
Water	3	1	1	3
Sewage	1	4	4	1
Urine	2	1	1	2
Egg powder	0	3	3	0

sorbite, raffinose, saccharose, maltose, adonite; Nos. 10 and 11, the power to ferment adonite and inulin; No. 12, the power to ferment adonite.

Of the organisms from human feces Nos. 1, 2, and 4 were varieties of B. coli-communior; No. 3 was a variety of B. coli-verus; Nos. 5 and 6 were varieties of B. acidi-lactici; No. 7 was B. coli-aerogenes; and Nos. 8, 9, and 10 were varieties of B. dysenteriae. Both the organisms from rabbit feces were varieties of B. coli-communior.

Of the water colon bacilli No. 3 had lost the power to ferment dulcite and adonite; No. 4 the power to ferment lactose, raffinose, dextrin, mannite, and inulin. Of those from sewage, Nos. 2 aud 5 were alkaligenes types.

The classification according to saccharose-fermentation alone shows a greater correlation with source than do classifications by means of saccharose and dulcite.

Correlation between Voges Proskauer and Methyl Red Reactions.—The 22 cultures that were alkaline to methyl red gave the Voges-Proskauer reaction, while all the cultures that were acid to methyl red did not. From Table 6 it will be seen that a definite relation exists between the source and the Voges-Proskauer and methyl-red reactions. Of the milk cultures 13, or 72%, gave the Voges-Proskauer reaction and all of these were alkaline to methyl red; of the cultures from human feces none gave the Voges-Proskauer reaction and none were alkaline to methyl red; of the rabbit feces none gave the Voges-Proskauer reaction nor was any one alkaline to methyl red; of the water cultures only 1, or 25%, gave the Voges-Proskauer reaction and it was alkaline to methyl red, while of the sewage cultures 4, or 80%, gave the reaction and were alkaline to methyl red; of the cultures from egg powder all 3 gave the Voges-Proskauer reaction and were alkaline to methyl red; of the cultures from human urine only 1 gave this reaction and it was alkaline to methyl red. These findings agree with those of Clark and Lubs and Levine that methyl-red-negative organisms are probably of nonfecal origin.

SUMMARY

A study of 45 organisms isolated from milk, human feces, rabbit feces, water, sewage, urine, and egg powder shows that—

There is a better correlation between saccharose-fermentation and source than between saccharose-dulcite-fermentation and source.

The methyl-red and Voges-Proskauer reactions are related, as suggested by Levine. All organisms alkaline to methyl red gave a positive Voges-Proskauer reaction, and all organisms acid to methyl red gave a negative Voges-Proskauer reaction.

Organisms that gave a positive Voges-Proskauer reaction were not found in feces and hence it seems that this and the methyl-red reactions are of sanitary importance in determining colon organisms of fecal origin.